(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 July 2002 (04.07.2002)

PCT

(10) International Publication Number
WO 02/051395 A1

(51) International Patent Classification[6]: A61K 31/045

(21) International Application Number: PCT/US99/11840

(22) International Filing Date: 28 May 1999 (28.05.1999)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/086,200 28 May 1998 (28.05.1998) US

(71) Applicant *(for all designated States except US)*: **CODON PHARMACEUTICALS, INC.** [US/US]; 207 Perry Parkway, Gaithersburg, MD 20877 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **BROWN, David, A.** [US/US]; 255 Hillcreast Drive, Seaford, NY 11783 (US). **REN, Wu, Yun** [US/US]; 12518 Timber Hollow Place, Germantown, MD 20874 (US).

(74) Agent: **TARCZA, John, E.**; Codon Pharmaceuticals, Inc., 207 Perry Parkway, Gaithersburg, MD 20877 (US).

(81) Designated States *(national)*: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/051395 A1

(54) Title: TREATMENT OF NEURODEGENERATIVE DISEASES

(57) Abstract: Disclosed are methods and compositions for increasing the differentiation of mammalian neuronal cells for purposes of treating neurodegenerative diseases or nerve damage by administration of various compounds, including alcohols, diols and/or triols and their analogues.

TITLE OF THE INVENTION
TREATMENT OF NEURODEGENERATIVE DISEASES

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of PCT/US98/05346 filed March 18, 1998, which is a continuation-in-part of PCT/US97/16642 filed September 18, 1997, which is a continuation-in-part of application Serial No. 08/933,143 filed September 18, 1997, which is a continuation-in-part of application Serial No. 60/026,577 filed September 18, 1996, of application Serial No. 60/035,947 filed January 21, 1997, of application Serial No. 60/036,863 filed February 4, 1997, and of application Serial No. 60/048,597 filed June 4, 1997.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not Applicable

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to increasing the differentiation of mammalian neuronal cells for purposes of treating neurodegenerative diseases or nerve damage by administration of various compounds, including alcohols, diols and/or triols and their analogues.

2. Description of Related Art

The compositions which are the subject of the present invention have been found to increase the melanin content of mammalian melanocytes, increase pigmentation in the epidermis of a mammal, and treat or prevent various skin

and proliferative disorders. See U.S. application Serial No. 60/026,577 filed September 18, 1996; application Serial No. 60/035,947 filed January 21, 1997; application Serial No. 60/036,863 filed February 4, 1997, and application Serial No. 60/048,597 filed June 4, 1997. It has now been found that the present compositions may be used for treating neurodegenerative diseases or nerve damage.

SUMMARY OF THE INVENTION

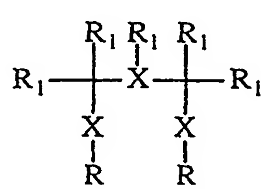
The present invention provides a method for increasing the differentiation of mammalian neuronal cells, which comprises administering to a mammal in need of such increase an effective amount of a C_3-C_{50} diol, which may be aliphatic or aromatic, linear, branched, mono-, bi- or polyclicic, saturated or unsaturated, unsubstituted, mono- or polysubstituted.

In another aspect, the present invention provides a composition for increasing the differentiation of mammalian neuronal cells, which comprises:

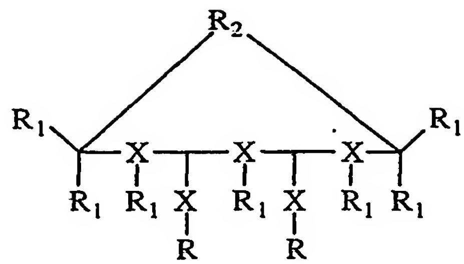
a) an effective amount of one or more compounds described above; and

b) a suitable carrier.

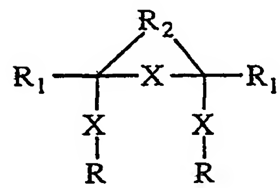
In yet another aspect, the present invention provides a method for increasing the differentiation of mammalian neuronal cells, which comprises administering to a mammal in need of such increase an effective amount of one or more compounds having the following structure:



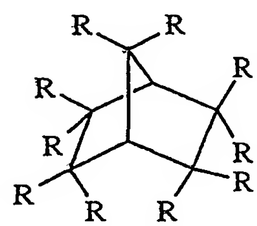
or



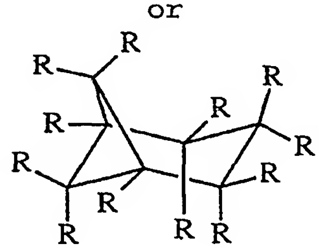
or



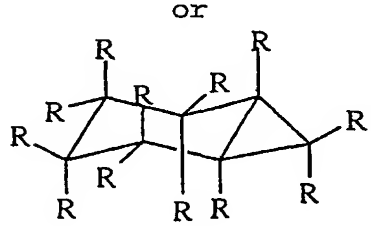
or



or



or



wherein

each **X** is independently selected from a single or double bond; or a group containing from one atom to twenty atoms, at least one of which is carbon, nitrogen, oxygen or sulfur;

each **R_1** is independently selected from hydrogen; halogen; an acyl or amino acyl group containing from one atom to twenty atoms, at least one of which is carbon, nitrogen, oxygen, or sulfur; or a group containing from one atom to twenty atoms, one of which is carbon, nitrogen, oxygen, or sulfur;

R_2 is a linear, branched or unbranched, cyclic, bicyclic or polycyclic group containing from one atom to fifty atoms, at least one of which is carbon, nitrogen, oxygen, or sulfur, and

each **R** is independently selected from **R_1**; **R_2**; hydroxyl, methyl, hydroxymethyl, $-(CH_2)_nCH_3-$, $-(CH_2)_nOH$, $-(CH_2)_nOR_1$, $-(CH_2)_n-CH(OH)-CHOH$, $-(CH_2)_n-CH(OH)-CH(OH)R_1$, $-(CH_2)_n-CH(OH)-(CH_2)_n-CH_2(OH)$, $-(CH_2)_n-CH(OH)-(CH_2)_n-CH(OH)R_1$ or $-CH_2OR_1$, wherein each n is independently an integer from 0-25;

and pharmaceutically acceptable salts or prodrugs thereof.

In another aspect, the present invention provides a composition for increasing the differentiation of mammalian neuronal cells, which comprises:

a) an effective amount of one or more compounds depicted above; and

b) a suitable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1B are printouts as described in Example 1.

Figures 2A-2D are printouts as described in Example 2.

Figures 3A-3D are printouts as described in Example 3.

DETAILED DESCRIPTION OF THE INVENTION

The compounds and compositions of the present invention effectively and efficiently increase differentiation of neuronal cells, including increased neuronal dendricity and neuronal tyrosine hydroxylase activity, which has several consequences. First, increasing dendricity leads to increased neuronal communication, thereby increasing neuronal function and performance. Thus, the present invention is useful for treating diseases or disorders marked by reduction of neuronal dendricity and function, including but not limited to Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, or any other neurodegenerative disease, or physical or toxic damage to brain, spinal or peripheral nerve cells. Further, the present invention is useful for restoring or optimizing neuronal communication, function or performance.

Second, increasing tyrosine hydroxylase activity directly increases dopamine synthesis. Thus, the present invention is particularly useful for treating Parkinson's disease which is specifically marked by depletion of dopamine synthesis.

Third, induction of neuronal differentiation reverses neuronal proliferative disorders. Thus, the present invention is useful for treating neuronal proliferative, tumorous, or cancerous disorders, or said disorders in any other cell type that might be similarly affected.

Finally, since the methods and compositions described herein induce differentiation, dendricity and tyrosine hydroxylase in a neuronal cell model, the present invention is useful for treating additional neurodegenerative disorders or neuropathies including but not limited to diffuse cerebral cortical atrophy, Lewy-body dementia, Pick disease, mesolimbocortical dementia, thalamic degeneration, Huntington chorea, cortical-striatal-spinal degeneration,

cortical-basal ganglionic degeneration, cerebrocerebellar degeneration, familial dementia with spastic paraparesis, polyglucosan body disease, Shy-Drager syndrome, olivopontocerebellar atrophy, progressive supranuclear palsy, dystonia musculorum deformans, Hallervorden-Spatz disease, Meige syndrome, familial tremors, Gilles de la Tourette syndrome, acanthocytic chorea, Friedreich ataxia, Holmes familial cortical cerebellar atrophy, Gerstmann-Straussler-Scheinker disease, progressive spinal muscular atrophy, progressive balbar palsy, primary lateral sclerosis, hereditary muscular atrophy, spastic paraplegia, peroneal muscular atrophy, hypertrophic interstitial polyneuropathy, heredopathia atactica polyneuritiformis, optic neuropathy, and ophthalmoplegia.

The active compounds according to the present invention are the C_3-C_{50} diols described above (by "diol" is meant a compound which has at least two, but permissibly more, -OH groups). Preferably, the active have one of the six structures depicted above. More preferably, **X** is independently selected from a single bond; or C_1-C_{10} alkylene, C_2-C_{10} alkenylene, or C_2-C_{10} alkynylene, each of which may contain one or more different heteroatoms or heteroatoms of the same type. More preferably each $\mathbf{R_1}$ is independently selected from hydrogen; fluoro; chloro; or C_1-C_{20} alkyl, C_2-C_{20} alkenyl, C_2-C_{20} alkynyl, C_7-C_{20} aralkyl, C_8-C_{20} aralkenyl, C_8-C_{20} aralkinyl, or C_6-C_{20} aryl, each of which may contain one or more different heteroatoms or heteroatoms of the same type, or carboxyl, carboxamido, carbalkoxy, sulfamido, sulfonamido; hydroxyl, or amino. More preferably $\mathbf{R_2}$ contains from two to twenty carbon atoms, each may contain one or more different heteroatoms or heteroatoms of the same type.

The preparation of the present compounds would be apparent to one of ordinary skill, and many of them are commercially available. Representative preferred compounds

include, but are not limited to:
1,2-Ethanediol
1,2-Propanediol (Propylene Glycol)
(S)-(+)-1,2-Propanediol [(S)-(+)-1,2-Propylene Glycol]
1,3-Propanediol
2,3-Dimethyl-2,3-Butanediol
2,3-Dimethyl-1,2-Butanediol
1-Phenyl-1,2-Propanediol
2-Methyl-1,3-Propanediol
1,2-Butanediol
1,3-Butanediol
1,4-Butanediol
2,3-Butanediol
(2R,3R)-(-)-2,3-Butanediol
(2S,3S)-(+)-2,3-Butanediol
2,3-meso-Butanediol
1,2-Pentanediol
1,4-Pentanediol
1,5-Pentanediol
2,4-Pentanediol
1,2-cis-cyclopentanediol
1,2-trans-cyclopentanediol
1,2-cis-cyclohexaneanediol
1,2-trans-cyclohexanediol
1,2-dihydroxy-4,5-cyclohexanediol carbonate
1,2,4,5-tetrahydroxycyclohexane
1,2-Hexanediol
1,5-Hexanediol
1,6-Hexanediol
2,5-Hexanediol
1,2-Heptanediol
1,7-Heptanediol
7-Octene-1,2-diol
1,2-Octanediol
1,8-Octanediol
1,2-Nonanediol

1,9-Nonanediol
1,2-Decanediol
1,10-Decanediol
1,2-Dodecanediol
1,12-Dodecanediol
1,2-Tetradecanediol
1,14-Tetradecanediol
1,2-Hexadecanediol
1,16-Hexadecanediol
Glycerol
1,2,4-Butanetriol
1,2,3-Trihydroxyhexane
1,2,6-Trihydroxyhexane
1,2,3-Heptanetriol
ß-estradiol
azabicyclo-(2,2,1)-heptanediol-3-one
1,4-dioxane-2,3-diol
5-norbornene-2,2-dimethanol
norbornane-2,2-dimethanol
2,3-norbornanediol (exo or endo or cis or trans)
2,3-cis-exo-norbornanediol
α-norborneol
2-norbornanemethanol
norbornane
borneol
camphor
camphene
camphane
norbornane acetic acid
norbornane-carboxylic acid
norbornane-dicarboxylic acid
2-endo-hexadecylamino-5-norbornene-2-exo-methanol
2-endo-hexadecylamino-5-norbornene-2,3-exo-dimethanol
2-(propyl-1,2-diol)-norbornane
1,2-dithiane-trans-4,5-diol
2,3-pyridinediol

2,3-pyridinediol hydrogen chloride
2,3-pyridinediol glycolic acid
2,3-dipyridyl-2,3-butanediol
2,2,4,4-tetramethyl-1,3-cyclobutanediol
norborneol
2,7-norbornanediol
2,5,7-norbornanetriol
2,6,7-norbornanetriol
2-hydroxy-2-norbornanemethanol
1-(exo-2-norbornyl-)-propan-1,2-diol
1-(endo-2-norbornyl-)-propan-1,2-diol
methyl-5-norbornene-2,3-dimethanol
2-norbornaneacetic acid
1,2-cis-cyclohexanedimethanol
3-cyclohexane-1,1-dimethanol
1,4-cyclohexanedimethanol
pentaerylthritol
pinane
pinaneol
2,3-cis/exo-pinanediol ([1R,2R,3S,5R]-[-]-pinanediol and [1S,2S,3R,5S]-[+]-pinanediol])
(1R)-(-)-trans-pinane-1,10-diol
(1S,2S,5S,)-2-hydroxy-3-pinanone
(-)-isopinocampheol
(S)-cis-verbenol
bornane
borneol
2,3-cis/exo-bornanediol
2,3-trans-bornanediol
camphanediol
camphenediol
cis-p-menthane-3,8-diol
trans-p-menthane-3,8-diol
sobrerol (trans-p-meth-6-ene-2,8-diol)
α-terpineol
terpinen-4-ol

(-)-cis-myrtanol [(1S,2R)-10-Pinanol]
(+)-trans-myrtanol [(1R,2R)-10-Pinanol]
(-)-trans-myrtanol [(1S,2S)-10-Pinanol]
(-)-myrtenal [(1R)-2-Pinen-10-al]
(-)-myrtenol [(1R)-2-Pinene-10-ol]
carveol [p-mentha-6,8-dien-2-one]
menthol

Particularly preferred compounds of this invention are 2,3-cis/exo-pinanediol ([1R,2R,3S,5R]-[-]-pinanediol and [1S,2S,3R,5S]-[+]-pinanediol]; 2,3-cis/exo-bornanediol; 5-norbornene-2,2-dimethanol; norbornane-2,2-dimethanol; 2-hydroxy-2-norbornanemethanol; 1-(exo-2-norbornyl-)-propan-1,2-diol; and 1-(endo-2-norbornyl-)-propan-1,2-diol. Other preferred compounds are (1S,2S,5S,)-2-hydroxy-3-pinanone; 2,3-trans-pinanediol; (1R)-(-)-trans-pinane-1,10-diol; 2,3-trans-bornanediol; cis-p-menthane-3,8-diol; trans-p-menthane-3,8-diol; 1,2-cis-cyclopentanediol, 2,3-cis/exo-norbornanediol; 2-norbornanemethanol; (1R)-(-)-myrtenol, and 3,3-dimethyl-1,2-butanediol.

The methods and compositions of the present invention contemplate the use of one or more of the above-mentioned compounds as an active ingredient for various uses. In a preferred embodiment, the active ingredient(s) is given orally, intravenously, or transdermally in an acceptable formulation. A particularly preferred carrier for some formulations is 1,2-propylene glycol since it is an excellent solvent for certain compounds in this invention including but not limited to 5-norbornene-2,2-dimethanol, 5-norbornane-2,2-dimethanol and 3,3-dimethyl-1,2-butanediol. Additionally, 1,2-propylene glycol as carrier has itself, as described in this invention, similar but lesser activity than the preferred active ingredient(s). Depending on the specific application, the compositions of the present invention may also include other active ingredients, as well as inert or inactive ingredients.

The dose regimen will depend on a number of factors which may readily be determined, such as severity and responsiveness of the condition to be treated, but will normally be one or more doses per day, with a course of treatment lasting from several days to several months, or until a cure is effected or a diminution of disease state is achieved. One of ordinary skill may readily determine optimum dosages, dosing methodologies and repetition rates. In general, it is contemplated that unit dosage form compositions according to the present invention will contain from about 0.01 mg to about 100 mg of active ingredient, preferably about 0.1 mg to about 10 mg of active ingredient. Topical formulations (such as creams, lotions, solutions, *etc.*) may have a concentration of active ingredient of from about 0.01% to about 50%, preferably from about 0.1% to about 10%.

The use of and useful and novel features of the present methods and compositions will be further understood in view of the following non-limiting examples.

<u>Example 1</u>

The PC12 rat pheochromocytoma cell line was obtained from American Type Culture Collection (ATCC). Cells were cultured in 85% RPMI 1640 medium, 10% horse serum (heat inactivated at 56°C for 30 minutes, 5% fetal bovine serum, 25 U/ml penicillin, and 25 ug/ml streptomycin (Greene, *et al.*, 1991, "Methodologies for the culture and experimental use of the rat PC12 rat pheochromocytoma cells line", pp. 207-225, In: Culturing Nerve Cells, The MIT Press, Cambridge, Massachusetts). Cells were cultured directly on plastic dishes at 37°C in 5% CO_2 in a humidified incubator.

PC12 rat pheochromocytoma cells are considered to be an excellent model for neuronal cells because they respond to treatment with nerve growth factor (NGF) by acquisition of a number of properties of neurons including cessation of

proliferation, extension of neurons, acquisition of electrical excitability, and increased neurotransmitter synthesis (Greene, *et al.*, 1991 and references therein). In addition, PC12 cells are used as a model for studies of prevention or cure of neurodegenerative diseases since they provide a robust screen for agents that maintain neuron survival and prevent neuron cell death in serum-free media (Rukenstein, *et al.*, 1991, *J. Neurosci.* 11:255-2563). Agents are considered to be potentially useful for treatment of neurodegenerative disorders if they not only promote PC12 cell survival, but also increase neurite outgrowth (Rukenstein, *et al.*, 1991). Agents are considered to be particularly useful for treatment of neurodegenerative disorders if they promote PC12 cell survival and neurite outgrowth in the absence of "priming" with NGF (Rukenstein, *et al.*, 1991). By virtue of their ability to express tyrosine hydroxylase and thereby synthesize dopamine, PC12 cells are considered to be an especially good model for studies of Parkinson's disease (Michel, *et al.*, 1994, *Europ. J. Neurosci. Assoc.* 6:577-586 and references therein). In addition, neurite outgrowth in PC12 cells has been used to identify agents that stimulate the regeneration of severed neuronal axons in the peripheral nerves of adult mammals (Sandrock, A. W. and Matthew, W. D., 1987, *Proc. Natl. Acad. Sci. U.S.A.* 84:6934-6938). Moreover, PC12 cells have been used as a model to study aspects of Alzheimer's disease (Shen, *et al.*, 1995, *Brain Res.* 671:282-292), amyotrophic lateral sclerosis (Durham, *et al.*, 1995, *Clin. Exp. Pharmacol. Physiol.* 22:366-67), Down's syndrome (Groner, *et al.*, 1994, *Biomed. Pharmacother.* 48:231-240), and age-related neurodegeneration (Taglialatela, *et al.*, 1996, *J. Neurochem.* 66:1826-1835).

For testing compounds for induction of dendricity (neurite outgrowth) and tyrosine hydroxylase activity in this invention, cells were plated at 15,000 cells/35 mm

dish. Two days following plating, cell culture media was replaced with that containing treatments. One week later, media and treatments were replaced with fresh media and treatments. Two weeks following the initial treatments, cells were examined microscopically, and the portion of cells exhibiting dendricity was estimated. Cells were harvested by trypsinization and counted by Coulter Counter. Cells were pelleted by centrifugation at 200 X g, and cell pellets were lysed in 600 ul 50 mM Tris/Acetate pH 6.0/0.2% Triton X-100 by vortexing, sonicating 5 seconds, incubating on ice for 30 minutes, followed by revortexing. Protein was determined on aliquots of cell lysate by the Bradford Coomassie Blue method (Bradford, 1967, *Anal. Biochem.* 72:248-254) using Bio-Rad Protein Assay Kit I. Tyrosine hydroxylase activity was determined by incubating 100 ul of PC12 cell lysate with 100 ul of the following reaction mixture at 37°C for 15 min: 200 mM sodium acetate pH 6.0, 50 uM tyrosine, 2000 U Cat/ml, 50 mU dihydropteridine reductase/ml, 0.1 mM NADH final, 200,000 cpm 3H tyrosine/100 ul, 0.1 mM NSD1015 (3-hydroxybenzylhydrazine), and 100 uM tetrahydrobiopterin (BH4) (Nagatsu, *et al.*, 1969, *Anal. Biochem.* 9:122-126; Ribeiro, *et al.* 1991, *J. Biol. Chem.* 16207-16211). Reactions were stopped by addition of 200 ul 10% activated charcoal in 0.1N HCl and incubation on ice for 15 min. This mixture was centrifuged at 17,300 X g for 5 min, and 200 ul supernatant was then filtered through a 0.22 uM GV Durapore centrifugal filter unit (Millipore) by centrifuging at 17,300 X g for 5 min. Filtrate was added to 4 ml Fisher Plus scintillation fluid and counted on a Hewlett Packard scintillation counter. Tyrosine hydroxylase activity was measured as tritium release and was calculated as dpm/ug protein and dpm/10^3 cells per hour.

Microscopic examination showed that a large portion of PC12 cells treated with 5 mM 5-norbornene-2,2-dimethanol (5-NBene-2,2-DM) acquired dendritic processes (Table 1, and

compare untreated PC12 cells in Figure 1A with 5-NBene-2,2-DM treated PC12 cells in Figure 1B). Lesser increases of dendritic processes were noted following treatment with 3,3-dimethyl-1,2-butandiol (3,3-M-1,2-BD) or 1,2-propylene glycol (1,2-PG) (Table 1). The most notable increases of tyrosine hydroxylase activity resulted from treatment with 25 mM 3,3-M-1,2-BD and 5 mM 5-NBene-2,2-DM (Table 1). Treatment with 1,2-PG, 3,3-M-1,2-BD and 5-NBene-2,2-DM increased the amount of protein per cells, a feature often associated with induction of differentiation. Increases of protein per cells were manifested morphologically as an increase in cell size (compare untreated PC12 cells in Figure 1A with 5-NBene-2,2-DM treated PC12 cells in Figure 1B). Examination of the data in Table 1 shows that increases of tyrosine hydroxylase per cell as a result of treatment with 1,2-PG, 3,3-M-1,2-BD or 5-NBene-2,2-DM, were in part, a result of increases of the amount of protein per cell. Ethanol (ETOH), used as a solvent for 3,3-M-1,2-BD and 5-NBene-2,2-DM, and IBMX (3-isobutly-1-methylxanthine), which increases cellular cAMP levels, resulted in only minor effects relative to the agents of this invention.

Table 1

	Cells/Dish ($X10^3$)	% Dendritic	ug Protein/ 10^3 Cells	dpm/hr: Tyrosine Hydroxylase /10^3 Cells	dpm/hr: Tyrosine Hydroxylase /ug Protein
Untreated	0.728	1%	0.47	3708	7888
Untreated	0.490	1%	0.61	4812	7888
Mean Untreated	0.609	1%	0.54	4260 (1.00X)	7888 (1.00X)
17 mM ETOH (0.1%)	0.410	2%	0.78	7344 (1.72X)[1]	9416 (1.19X)
85 mM ETOH (0.5%)	0.367	5%	0.82	7308 (1.72X)	8912 (1.13X)
100 mM 1,2-PG	0.180	10%	1.66	12988 (3.05X)	7824 (0.99X)
300 mM 1,2-PG	0.197	2%	1.57	16152 (3.79X)	10288 (1.30X)
10 mM 3,3-M-1,2-BD	0.214	25%	1.11	8828 (2.07X)	7952 (1.01X)
25 mM 3,3-M-1,2-BD	0.044	5%	2.22	37148 (8.72X)	16732 (2.12X)
5 mM 5-NBene-2,2-DM	0.155	50%	1.64	28956 (6.80X)	17656 (2.23X)
10 mM 5-NBene-2,2-DM	0.010	25%	2.33	12732 (3.00X)	5464 (0.69X)
0.1 mM IBMX	0.346	2%	1.20	9148 (2.15X)	7624 (0.97X)

[1]Fold increase relative to mean untreated control value.

The reduced cell numbers resulting from treatment with 1,2-PG, 3,3-M-1,2-BD or 5-NBene-2,2-DM are in part indicative of the differentiation process induced by treatments. However, in the case of treatment with 25 mM 3,3-M-1,2-BD and 10 mM 5-NBene-2,2-DM, some cells detached concomitantly with the acquisition of dendricity that occurred earlier than for other treatments. This detachment phenomenon has been noticed previously for PC12 cells induced to differentiate with NGF, and can be avoided by coating treatment dishes with collagen (reviewed in Greene, *et al.*, 1991). Treatment with collagen also shortens the time required for dendrite formation and greatly increases the extent of dendrite formation in response to treatment with NGF (reviewed in Greene, *et al.*, 1991). Thus, it is contemplated that the compounds of this invention will prove to exhibit more activity when tested on collagen-coated dishes.

Induction of differentiation as indicated by induction of dendricity, induction of tyrosine hydroxylase activity, increased cellular protein levels and induction of cell cycle arrest as indicated by reduced growth, indicate that the compounds of this invention can act as chemotherapeutic agents for treatment of neural tumorous and cancerous disorders and additional neural proliferative disorders. In addition, it is contemplated that the compounds of this invention will treat tumorous, cancerous and proliferative disorders arising from additional cell types.

It should be particularly noted that the compounds of this invention induced dendricity and tyrosine hydroxylase activity in the absence of priming with NGF, a prerequisite for induction of neurite extension by many other agents tested on PC12 cells (Steiner, *et al.* 1997, *Nature Medicine* 3:421-428; Rukenstein, *et al.* 1991, *J. Neurosci.* 11:2552-2563). Several agents under consideration as treatments for neurodegenerative diseases do not promote neurite extension even in NGF-primed PC12 cells (*e.g.*,

IGF-I and IGF-II; Rukenstein, *et al.*, 1991 and references therein). Moreover, many agents under consideration for treatment of neurodegenerative diseases including GDNF (glial cell-derived neurotrophic factor) being developed for treatment of Parkinson's disease are neurotrophic peptides that cannot cross the blood-brain barrier and therefore require gene therapy implantation at the site of action (Haase, *et al.* 1997, *Nature Medicine* 3:429-436). Furthermore, L-Dopa which is presently used for treatment of Parkinson's disease is toxic (Yahr, M. D. 1993, *Adv. Neurol.* 60:11-17), in part, by generation of peripherally formed dopamine (Riederer, *et al.* 1993, *Adv. Neurol.* 60:626-635), and in part, by virtue of its ability to form highly reactive semiquinone and quinones via autooxidation (Karg, *et al.* 1989, *Acta Derm. Venereol.* 69:521-524). Given that the agents of the present invention: (i) act directly without a requirement for NGF; (ii) induce neuronal differentiation thereby setting into motion cellular reprogramming to the desired phenotype; (iii) induce tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis; (iv) are small molecule drugs that are likely to cross the blood brain barrier; and (v) have no known ability to form semiquinone, quinone or other toxic intermediates, it is contemplated that the agents of this invention will be particularly advantageous for treatment of neurodegenerative diseases including but not limited to Parkinson's disease.

Example 2

Previous studies have shown that both induction of tyrosine hydroxylase activity and neurite outgrowth in PC12 cells are mediated by the nitric oxide (NO)/guanosine 3',5'-cyclic monophosphate (cGMP) signal transduction pathway (Roskoski and Roskoski, 1987, *J. Neuochem.* 48:236-242; Hindley, *et al.*, 1997, *J. Neurosci. Res.* 47:427-439). These studies have employed NO donors such as sodium nitroprusside to induce neurite outgrowth and tyrosine hydroxylase activity, and the guanylyl cyclase inhibitor LY83583 to block these effects (Roskoski and Roskoski, 1987, *supra;* Hindley, *et al.*, *supra*). In contrast, the compounds of this invention do not donate NO, but rather induce treated cells to produce NO, with concomitant induction of tyrosine hydroxylase activity and cGMP-dependent neurite outgrowth.

PC12 cells were grown and treated with 5-norbornene-2,2-dimethanol as described in Example 1, except that cells were analyzed one week after treatments, rather than two weeks after treatments. Furthermore, in addition to the analysis described in Example 1, the media of cells was also collected for analysis of nitric oxide (NO). For measurement of NO, media was centrifuged at 200 X g for 5 min to remove cells and debris. Nitric oxide was measured using a CalBiochem (San Diego, CA) Colorimetric Nitric Oxide Assay Kit. Since nitric oxide is converted into nitrite and nitrate with seconds of production in biological fluids, nitric oxide is measured by first converting nitrate to nitrite using nitrate reductase, followed by addition of Greiss reagent to detect nitrite as optical density at 550 nm (Moshage, *et al.*, 1995, *Clin. Chem.* 41:892-896; Schmidt, *et al.*, 1995, *Biochemica* 2:22)).

Results show that the degree of neurite outgrowth (% with neurites of length exceeding cell body diameter), the amount of nitric oxide (NO) released into the media, the

amount of NO generated per cell, and tyrosine hydroxylase (Tyr Hydrox) activity all increased following treatment with 5-norbornene-2,2-dimethanl (5-NBene-2,2-DM)(Table 2). Induction of NO and tyrosine hydroxylase were apparent at 1 mM 5-NBene-2,2-DM, and appeared to reach a maximum at 2.5 mM 5-NBene-2,2-DM, since no further increases were observed at 5 mM 5-NBene-2,2-DM (Table 2). In contrast, neurite outgrowth was much greater at 5 mM 5-NBene-2,2-DM than at 2.5 mM 5 mM 5-NBene-2,2-DM (Table 2) than following two weeks treatment (Table 1 and Figure 2). Figure 2 shows photographs of cultured unstained PC12 cells. In Figure 2A, PC12 cells are untreated. PC12 cells in Figure 2B were treated for one week with 2.5 mM 5-NBene-2,2-DM, while cells in Figures 2C and 2D were treated with 5 mM 5-NBene-2,2-DM. PC12 cells treated with 5 mM 5-NBene-2,2-DM exhibited both longer and more branched neurites than those treated with 2.5 mM 5-NBene-2,2-DM.

Table 2

	% With Neurites[1]	NO uM	nmole NO/ 10^6 Cells	Tyr Hydrox dpm/hr/10^3
Control	5%	4.9	4.1	4244
Control	5%	6.9	8.0	4372
	5% (1.0X)	5.9 (1.0X)	6.1 (1.0X)	4308 (1.0X)
85 mM ETOH	1%	4.0	3.7	4393
85 mM ETOH	1%	ND[2]	ND	4352
	1% (0.2X)	2.0 (0.3X)	1.9 (0.3X)	4373 (1.0X)
1 mM 5-NBene-2,2-DM	10%	11.2	11.4	5081
1 mM 5-NBene-2,2-DM	10%	7.5	8.7	5223
	10%(2.0X)	9.4 (1.6X)	10.1(1.7X)	5152 (1.2X)
2.5 mM 5-NBene-				

2,2-DM	20%	15.4	27.5	8968
2.5 mM 5-NBene-				
2,2-DM	<u>20%</u>	<u>17.8</u>	<u>36.0</u>	<u>10749</u>
	20%(4.0X)	16.6(2.8X)	31.8(5.2X)	9859(2.3X)
5 mM 5-NBene-				
2,2-DM	50%	12.8	37.1	9417
5 mM 5-NBene-				
2,2-DM	<u>50%</u>	<u>12.3</u>	<u>32.8</u>	<u>10479</u>
	50%(10X)	12.6(2.1X)	35.0(5.7X)	9948(2.3X)

[1]Values are estimates wherein neurites were counted as extended if their length exceeded the cell body's diameter. Evaluations include single cells and cells on periphery of clumps, but exclude cells in middle of clumps.

[2]ND: Not Detectable.

Previous studies have shown that induction of tyrosine hydroxylase activity and neurite outgrowth in PC12 cells by NO donors occurs by the NO/cGMP/PKG (protein kinase G) pathway (Roskoski and Roskoski, *supra*; Hindley, *et al.*, *supra*). However, 5-NBene-2,2-DM contains no nitrogen, and as such, cannot act as a NO donor. Rather, the results in Table 2 show that 5-NBene-2,2-DM induces synthesis of NO within PC12 cells.

<u>Example 3</u>

To further elucidate and substantiate the role of NO in differentiation of PC12 cells, additional studies were done using the guanylyl cyclase inhibitor LY83583. This inhibitor blocks differentiation of PC12 cells otherwise induced by NO donors, indicating they act via cGMP. Similarly it was contemplated that if 5-NBene-2,2-DM was inducing NO within PC12 cells, and if this endogenously

generated NO was responsible for inducing differentiation of PC12 cells by the cGMP pathway, then LY83583 should block the effects of 5-NBene-2,2-DM. All PC12 culture and treatment methods were the same as in Examples 1 and 2.

Co-treatment of PC12 cells with 5-NBene-2,2-DM and the guanylyl cyclase inhibitor LY83583 shows that blockage of the NO/cGMP/PKG pathway completely blocks neurite outgrowth otherwise induced by 5-NBene-2,2-DM. Figure 3A shows photographs of unstained cultured PC12 cells. Figure 3B shows induction of extensive neurite outgrowth in PC12 cells treated with 5 mM 5-NBene-2,2-DM for two weeks. Figure 3D shows that PC12 cells cotreated with both 5 mM 5-NBene-2,2-DM and 0.1 uM LY83583 for two weeks exhibit no neurite outgrowth, similar to untreated PC12 cells (Figure 3A) or PC12 cells treated with 0.1 uM LY83583 alone for two weeks (Figure 3C).

Results shown here and in Example 2 show that 5-NBene-2,2-DM induces nitric oxide activity, that induction of nitric oxide activity is associated with induction of neurite outgrowth and tyrosine hydroxylase activity, and that LY83583 can block neurite outgrowth induced by 5-NBene-2,2-DM. These results indicate that 5-NBene-2,2-DM stimulates PC12 cell differentiation by stimulating the NO/cGMP/PKC signal transduction pathway. Since, 5-NBene-2,2-DM cannot be a source of NO, 5-NBene-2,2-DM must stimulate NO production within PC12 cells. Furthermore, this endogenously generated NO must act via stimulating cGMP production, since the effects of 5-NBene-2,2-DM on PC12 cells differentiation are blocked by LY83583.

The compounds of the present invention are distinguished from NO donors not only by the fact that they induce synthesis of NO within treated cells, but also because in contrast to NO donors (Hindley *et al.*, *supra*), the compounds of the present invention induce neurite outgrowth from PC12 cells in the absence of co-treatment with NGF. Thus, unlike NO donors which induce no neurite

outgrowth in the absence of NGF (Hindley *et al.*, *supra*), the compounds of the present invention induce neurite outgrowth in the complete absence of added NGF. In fact, as shown below in Example 4, addition of NGF has little or no effect on neurite outgrowth induced by compounds of the present invention.

Although previous studies have shown that NO donors augment the effects of NGF on PC12 cell differentiation, studies have also shown that treatment of PC12 cells or neurons with NO donors in the absence of NGF leads to morphological changes associated with neurodegeneration (Hess, *et al.*, 1993, *Nature* 366:562-565). Thus, the compounds of the present invention must induce neurite outgrowth by affecting signal transduction pathways in addition to the NO/cGMP/PKG pathway, or this pathway must be modulated differently by endogenously produced NO than by NO from exogenous donors. Therefore, although stimulation of differentiation of PC12 cells by the compounds of the present invention requires activation of the NO/cGMP/PKG pathway, additional effects on cell function must occur, some of which may coincide with the effects of NGF.

Both 5-NBene-2,2-DM and 2,3-cis/exo-pinanediol have been shown to stimulate NO production and differentiation of a melanoma cell line. Many other diols, including those shown in Example 1, Table 1, have been shown to induce differentiation of melanoma cells and melanocytes. Since melanocytes are considered to be a good model for elucidating biological modulators of neuronal cells, it is contemplated that other diols including but not limited to 5-NBene-2,2-DM and 2,3-cis/exo-pinanediol will induce NO production and differentiation of PC12 cells. Since many different types of diols, and some related alcohols and triols, similarly induce differentiation of melanoma cells and melanocytes, it is contemplated that many or all of these will be active as inducers of PC12 and neuronal cell

differentiation. It is further contemplated that similar to 5-NBene-2,2-DM, many of these diols will stimulate PC12 cell and neuronal cell differentiation, at least in part, via the NO/cGMP/PKG pathway.

Example 4

As discussed in Example 1, the compounds of the present invention appear to be particularly efficacious relative to other compounds proposed for treatment of neurodegenerative diseases (Steiner, *et al.*, 1997, *Nature Med.* 3:421-428; Rukenstein, *et al.*, 1991, *J. Neurosci.* 11:2552-2563) in that they act without co-treatment with nerve growth factor (NGF). Furthermore, as discussed in Example 3, although the compounds of the present invention induce PC12 differentiation at least in part via the NO/cGMP/PKG pathway, NO donors are ineffective without the presence of NGF. The purpose of this study was to determine if addition of NGF would markedly improve the ability of the compounds of this invention to stimulate PC12 differentiation.

For the studies shown in Table 3, PC12 cells were treated with the bicyclic diol 5-norbornene-2,2-dimethanol (5-NBene-2,2-DM), the bicyclic diol 2,3-cis/exo-pinanediol (2,3-cs/ex-PD), or the bicyclic alcohol (S)-cis-verbenol (S-cs-VBol). The concentration of NGF used for co-treatments (0.5 ng/ml) was identical to that shown to be essential for induction of PC12 cell differentiation by immunophilins (Steiner, *et al.*, *supra*). 50 ng/ml NGF was used as a positive control (Steiner, et al., *supra*). Cells were plated, treated and neurite outgrowth evaluated as described in Example 2, Table 1 except that cells were evaluated 7 and 21 days after initiation of treatments.

Results showed that supplementary NGF resulted in a slight stimulation of neurite outgrowth when PC12 cells were examined 7 days after the initiation of treatments, but mixed results were obtained when cells were examined 21

days after the initiation of treatments (Table 3). The highest levels of neurite outgrowth were seen following 7 days treatment with 50 ng/ml NGF, following 7 days treatment with 5 mM 2,3-cs/ex-PD, and following 21 days treatment with 5 mM 5-NBene-2,2-DM (Table 3); highest levels of neurite outgrowth were apparently unaffected by inclusion of 0.5 ng/ml NGF. Results show that the compounds of this invention can induce significant levels of neurite outgrowth (similar to the 50 ng/ml NGF positive control) without inclusion of supplementary NGF. Furthermore, results show that although the bicyclic alcohol S-cis-Verbenol (S-cs-VBol) induced some neurite outgrowth, it was much less effective than the bicyclic diols examined here.

Table 3

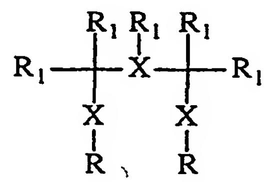
	% with Extended Neurites[1]			
	7 Day		21 Day	
	No NGF	0.5 ng/ ml NGF	No NGF	0.5 ng/ ml NGF
Untreated	<1	<1	5	10
85 mM ETOH	<1	<1	2	2
50 ng/ml NGF	50	50	25	25
500 uM 5-NBene-2,2-DM	<1	1	5	10
1 mM 5-NBene-2,2-DM	1	2	15	5
2.5 mM 5-NBene-2,2-DM	2	3	15	5
5 mM 5-NBene-2,2-DM	5	10	50	50
7.5 mM 5-NBene-2,2-DM	10	15	25	50

100 uM 2,3-cs/ ex-PD	<1	<1	2	2
500 uM 2,3-cs/ ex-PD	<1	1	5	10
1.mM 2,3-cs/ ex-PD	1	5	25	20
2.5 mM 2,3-cs/ ex-PD	7	10	25	10
5 mM 2,3-cs/ ex-PD	50	50	10	10
100 uM S-cs-VBol	<1	1	5	10
500 uM S-cs-VBol	<1	2	5	20
1 mM S-cs-VBol	1	2	5	5
2.5 mM S-cs-VBol	2	3	10	10

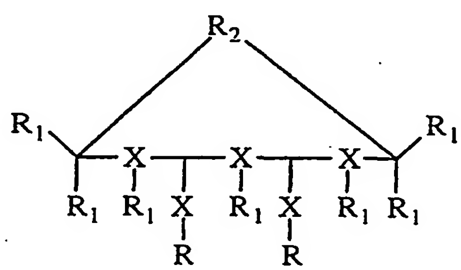
[1]Values are estimates wherein neurites were counted as extended if their length exceeded the cell body's diameter. Evaluations include single cells and cells on periphery of clumps, but exclude cells in middle of clumps.

CLAIMS

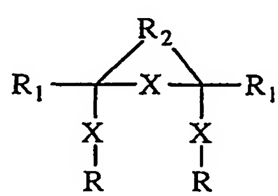
1. A method for increasing the differentiation of mammalian neuronal cells, which comprises administering to a mammal in need of such increase an effective amount of one or more compounds having the following structure:



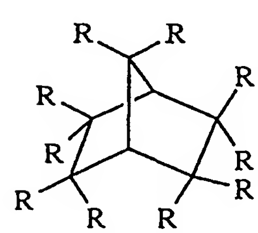
or



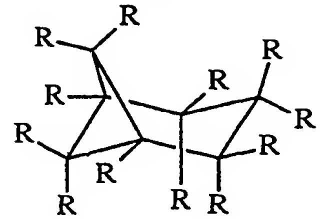
or



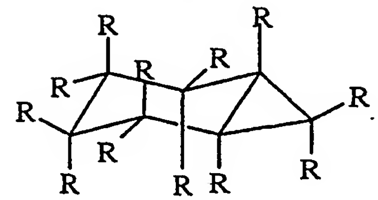
or



or



or



wherein

each **X** is independently selected from a single or double bond; or a group containing from one atom to twenty atoms, at least one of which is carbon, nitrogen, oxygen or sulfur;

each $\mathbf{R_1}$ is independently selected from hydrogen; halogen; an acyl or amino acyl group containing from one atom to twenty atoms, at least one of which is carbon, nitrogen, oxygen, or sulfur; or a group containing from one atom to twenty atoms, one of which is carbon, nitrogen, oxygen, or sulfur;

$\mathbf{R_2}$ is a linear, branched or unbranched, cyclic, bicyclic or polycyclic group containing from one atom to fifty atoms, at least one of which is carbon, nitrogen, oxygen, or sulfur, and

each **R** is independently selected from $\mathbf{R_1}$; $\mathbf{R_2}$; hydroxyl, methyl, hydroxymethyl, $-(CH_2)_nCH_3-$, $-(CH_2)_nOH$, $-(CH_2)_nOR_1$, $-(CH_2)_n-CH(OH)-CHOH$, $-(CH_2)_n-CH(OH)-CH(OH)R_1$, $-(CH_2)_n-CH(OH)-(CH_2)_n-CH_2(OH)$, $-(CH_2)_n-CH(OH)-(CH_2)_n-CH(OH)R_1$ or $-CH_2OR_1$, wherein each n is independently an integer from

0-25;

and pharmaceutically acceptable salts or prodrugs thereof.

2. The method of claim 1, wherein

X is independently selected from a single bond; or C_1-C_{10} alkylene, C_2-C_{10} alkenylene, or C_2-C_{10} alkynylene, each of which may contain one or more different heteroatoms or heteroatoms of the same type;

each $\mathbf{R_1}$ is independently selected from hydrogen; fluoro; chloro; or C_1-C_{20} alkyl, C_2-C_{20} alkenyl, C_2-C_{20} alkynyl, C_7-C_{20} aralkyl, C_8-C_{20} aralkenyl, C_8-C_{20} aralkinyl, or C_6-C_{20} aryl, each of which may contain one or more different heteroatoms or heteroatoms of the same type, or carboxyl, carboxamido, carbalkoxy, sulfamido, sulfonamido; hydroxyl, or amino;

each $\mathbf{R_2}$ contains from two to twenty carbon atoms, each may contain one or more different heteroatoms or heteroatoms of the same type.

3. The method of claim 1, wherein the compound is selected from the group consisting of 1,2-Ethanediol, 1,2-Propanediol (Propylene Glycol), (S)-(+)-1,2-Propanediol [(S)-(+)-1,2-Propylene Glycol], 1,3-Propanediol, 2,3-Dimethyl-2,3-Butanediol, 2,3-Dimethyl-1,2-Butanediol, 1-Phenyl-1,2-Propanediol, 2-Methyl-1,3-Propanediol, 1,2-Butanediol, 1,3-Butanediol, 1,4-Butanediol, 2,3-Butanediol, (2R,3R)-(-)-2,3-Butanediol, (2S,3S)-(+)-2,3-Butanediol, 2,3-meso-Butanediol, 1,2-Pentanediol, 1,4-Pentanediol, 1,5-Pentanediol, 2,4-Pentanediol, 1,2-cis-cyclopentanediol, 1,2-trans-cyclopentanediol, 1,2-cis-cyclohexaneanediol, 1,2-trans-cyclohexanediol, 1,2-dihydroxy-4,5-cyclohexane-diol carbonate, 1,2,4,5-tetrahydroxycyclohexane, 1,2-Hexanediol, 1,5-Hexanediol, 1,6-Hexanediol,

2,5-Hexanediol, 1,2-Heptanediol, 1,7-Heptanediol, 7-Octene-1,2-diol, 1,2-Octanediol, 1,8-Octanediol, 1,2-Nonanediol, 1,9-Nonanediol, 1,2-Decanediol, 1,10-Decanediol, 1,2-Dodecanediol, 1,12-Dodecanediol, 1,2-Tetradecanediol, 1,14-Tetradecanediol, 1,2-Hexadecanediol, 1,16-Hexadecanediol, Glycerol, 1,2,4-Butanetriol, 1,2,3-Trihydroxyhexane, 1,2,6-Trihydroxyhexane, 1,2,3-Heptanetriol, ß-estradiol, azabicyclo-(2,2,1)-heptanediol-3-one, 1,4-dioxane-2,3-diol, 5-norbornene-2,2-dimethanol, norbornane-2,2-dimethanol, 2,3-norbornanediol (exo or endo or cis or trans), 2,3-cis-exo-norbornanediol, α-norborneol, 2-norbornanemethanol, norbornane, borneol, camphor, camphene, camphane, norbornane acetic acid, norbornane-carboxylic acid, norbornane-dicarboxylic acid, 2-endo-hexadecylamino-5-norbornene-2-exo-methanol, 2-endo-hexadecylamino-5-norbornene-2,3-exo-dimethanol, 2-(propyl-1,2-diol)-norbornane, 1,2-dithiane-trans-4,5-diol, 2,3-pyridinediol, 2,3-pyridinediol hydrogen chloride, 2,3-pyridinediol glycolic acid, 2,3-dipyridyl-2,3-butanediol, 2,2,4,4-tetramethyl-1,3-cyclobutanediol, norborneol, 2,7-norbornanediol, 2,5,7-norbornanetriol, 2,6,7-norbornanetriol, 2-hydroxy-2-norbornanemethanol, 1-(exo-2-norbornyl-)-propan-1,2-diol, 1-(endo-2-norbornyl-)-propan-1,2-diol, methyl-5-norbornene-2,3-dimethanol, 2-norbornaneacetic acid, 1,2-cis-cyclohexanedimethanol, 3-cyclohexane-1,1-dimethanol, 1,4-cyclohexanedimethanol, pentaerylthritol, pinane, pinaneol, 2,3-cis/exo-pinanediol ([1R,2R,3S,5R]-[-]-pinanediol, and [1S,2S,3R,5S]-[+]-pinanediol]), (1R)-(-)-trans-pinane-1,10-diol, (1S,2S,5S,)-2-hydroxy-3-pinanone, (-)-isopinocampheol, (S)-cis-verbenol, bornane, borneol, 2,3-cis/exo-bornanediol, 2,3-trans-bornanediol, camphanediol, camphenediol, cis-p-menthane-3,8-diol, trans-p-menthane-3,8-diol, sobrerol (trans-p-meth-6-ene-2,8-diol), α-terpineol, terpinen-4-ol, (-)-cis-myrtanol [(1S,2R)-10-Pinanol], (+)-trans-myrtanol [(1R,2R)-10-Pinanol], (-)-trans-myrtanol [(1S,2S)-10-

Pinanol], (-)-myrtenal [(1R)-2-Pinen-10-al], (-)-myrtenol [(1R)-2-Pinene-10-ol], carveol [p-mentha-6,8-dien-2-one] and menthol.

4. The method of claim 3, wherein the compound is selected from 2,3-cis/exo-pinanediol ([1R,2R,3S,5R]-[-]-pinanediol and [1S,2S,3R,5S]-[+]-pinanediol]; 2,3-cis/exo-bornanediol; 5-norbornene-2,2-dimethanol; norbornane-2,2-dimethanol; 2-hydroxy-2-norbornanemethanol; 1-(exo-2-norbornyl-)-propan-1,2-diol; and 1-(endo-2-norbornyl-)-propan-1,2-diol; (1S,2S,5S,)-2-hydroxy-3-pinanone; 2,3-trans-pinanediol; (1R)-(-)-trans-pinane-1,10-diol; 2,3-trans-bornanediol; cis-p-menthane-3,8-diol; trans-p-menthane-3,8-diol; 1,2-cis-cyclopentanediol, 2,3-cis/exo-norbornanediol; 2-norbornanemethanol; (1R)-(-)-myrtenol, and 3,3-dimethyl-1,2-butanediol.

5. The method of claim 1, wherein the differentiation reverses neuronal damage.

6. The method of claim 1, wherein the differentiation alleviates a neurodegenerative disease.

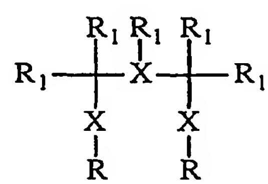
7. The method of claim 6, wherein the disease is selected from the group consisting of Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, diffuse cerebral cortical atrophy, Lewy-body dementia, Pick disease, mesolimbocortical dementia, thalamic degeneration, Huntington chorea, cortical-striatal-spinal degeneration, cortical-basal ganglionic degeneration, cerebrocerebellar degeneration, familial dementia with spastic paraparesis, polyglucosan body disease, Shy-Drager syndrome, olivopontocerebellar atrophy, progressive supranuclear palsy, dystonia musculorum deformans, Hallervorden-Spatz disease, Meige syndrome, familial tremors, Gilles de la Tourette syndrome, acanthocytic chorea, Friedreich ataxia,

Holmes familial cortical cerebellar atrophy, Gerstmann-Straussler-Scheinker disease, progressive spinal muscular atrophy, progressive balbar palsy, primary lateral sclerosis, hereditary muscular atrophy, spastic paraplegia, peroneal muscular atrophy, hypertrophic interstitial polyneuropathy, heredopathia atactica polyneuritiformis, optic neuropathy, and ophthalmoplegia.

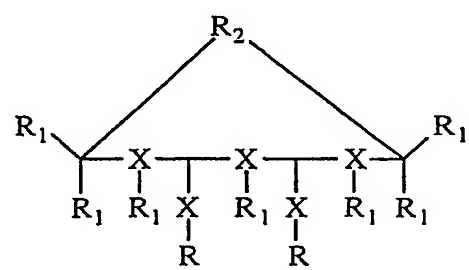
8. The method of claim 1, wherein the differentiation alleviates a cancerous, tumorous or proliferative disorder.

9. A composition for increasing the differentiation of mammalian neuronal cells, which comprises:

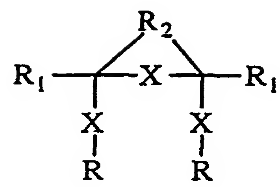
a) an effective amount of one or more compounds having the following structure:



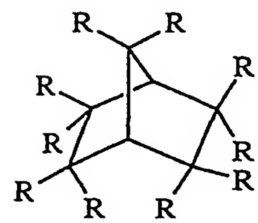
or



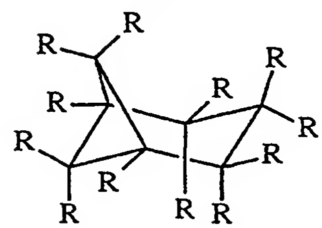
or



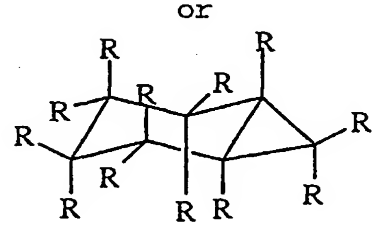
or



or



or



wherein

each **X** is independently selected from a single or double bond; or a group containing from one atom to twenty atoms, at least one of which is carbon, nitrogen, oxygen or sulfur;

each $\mathbf{R_1}$ is independently selected from hydrogen; halogen; an acyl or amino acyl group containing from one atom to twenty atoms, at least one of which is carbon, nitrogen, oxygen, or sulfur; or a group containing from one atom to twenty atoms, one of which is carbon, nitrogen,

oxygen, or sulfur;

R_2 is a linear, branched or unbranched, cyclic, bicyclic or polycyclic group containing from one atom to fifty atoms, at least one of which is carbon, nitrogen, oxygen, or sulfur, and

each R is independently selected from R_1; R_2; hydroxyl, methyl, hydroxymethyl, $-(CH_2)_nCH_3-$, $-(CH_2)_nOH$, $-(CH_2)_nOR_1$, $-(CH_2)_n-CH(OH)-CHOH$, $-(CH_2)_n-CH(OH)-CH(OH)R_1$, $-(CH_2)_n-CH(OH)-(CH_2)_n-CH_2(OH)$, $-(CH_2)_n-CH(OH)-(CH_2)_n-CH(OH)R_1$ or $-CH_2OR_1$, wherein each n is independently an integer from 0-25;

and pharmaceutically acceptable salts thereof; and

b) a suitable carrier.

10. The composition of claim 9, wherein

X is independently selected from a single bond; or C_1-C_{10} alkylene, C_2-C_{10} alkenylene, or C_2-C_{10} alkynylene, each of which may contain one or more different heteroatoms or heteroatoms of the same type;

each R_1 is independently selected from hydrogen; fluoro; chloro; or C_1-C_{20} alkyl, C_2-C_{20} alkenyl, C_2-C_{20} alkynyl, C_7-C_{20} aralkyl, C_8-C_{20} aralkenyl, C_8-C_{20} aralkinyl, or C_6-C_{20} aryl, each of which may contain one or more different heteroatoms or heteroatoms of the same type, or carboxyl, carboxamido, carbalkoxy, sulfamido, sulfonamido; hydroxyl, or amino;

each R_2 contains from two to twenty carbon atoms, each may contain one or more different heteroatoms or heteroatoms of the same type.

11. The composition of claim 9, wherein the compound is selected from the group consisting of 1,2-Ethanediol, 1,2-Propanediol (Propylene Glycol), (S)-(+)-1,2-Propanediol

[(S)-(+)-1,2-Propylene Glycol], 1,3-Propanediol, 2,3-Dimethyl-2,3-Butanediol, 2,3-Dimethyl-1,2-Butanediol, 1-Phenyl-1,2-Propanediol, 2-Methyl-1,3-Propanediol, 1,2-Butanediol, 1,3-Butanediol, 1,4-Butanediol, 2,3-Butanediol, (2R,3R)-(-)-2,3-Butanediol, (2S,3S)-(+)-2,3-Butanediol, 2,3-meso-Butanediol, 1,2-Pentanediol, 1,4-Pentanediol, 1,5-Pentanediol, 2,4-Pentanediol, 1,2-cis-cyclopentanediol, 1,2-trans-cyclopentanediol, 1,2-cis-cyclohexaneanediol, 1,2-trans-cyclohexanediol, 1,2-dihydroxy-4,5-cyclohexane-diol carbonate, 1,2,4,5-tetrahydroxycyclohexane, 1,2-Hexanediol, 1,5-Hexanediol, 1,6-Hexanediol, 2,5-Hexanediol, 1,2-Heptanediol, 1,7-Heptanediol, 7-Octene-1,2-diol, 1,2-Octanediol, 1,8-Octanediol, 1,2-Nonanediol, 1,9-Nonanediol, 1,2-Decanediol, 1,10-Decanediol, 1,2-Dodecanediol, 1,12-Dodecanediol, 1,2-Tetradecanediol, 1,14-Tetradecanediol, 1,2-Hexadecanediol, 1,16-Hexadecanediol, Glycerol, 1,2,4-Butanetriol, 1,2,3-Trihydroxyhexane, 1,2,6-Trihydroxyhexane, 1,2,3-Heptanetriol, ß-estradiol, azabicyclo-(2,2,1)-heptanediol-3-one, 1,4-dioxane-2,3-diol, 5-norbornene-2,2-dimethanol, norbornane-2,2-dimethanol, 2,3-norbornanediol (exo or endo or cis or trans), 2,3-cis-exo-norbornanediol, α-norborneol, 2-norbornanemethanol, norbornane, borneol, camphor, camphene, camphane, norbornane acetic acid, norbornane-carboxylic acid, norbornane-dicarboxylic acid, 2-endo-hexadecylamino-5-norbornene-2-exo-methanol, 2-endo-hexadecylamino-5-norbornene-2,3-exo-dimethanol, 2-(propyl-1,2-diol)-norbornane, 1,2-dithiane-trans-4,5-diol, 2,3-pyridinediol, 2,3-pyridinediol hydrogen chloride, 2,3-pyridinediol glycolic acid, 2,3-dipyridyl-2,3-butanediol, 2,2,4,4-tetramethyl-1,3-cyclobutanediol, norborneol, 2,7-norbornanediol, 2,5,7-norbornanetriol, 2,6,7-norbornanetriol, 2-hydroxy-2-norbornanemethanol, 1-(exo-2-norbornyl-)-propan-1,2-diol, 1-(endo-2-norbornyl-)-propan-1,2-diol, methyl-5-norbornene-2,3-dimethanol, 2-

norbornaneacetic acid, 1,2-cis-cyclohexanedimethanol, 3-cyclohexane-1,1-dimethanol, 1,4-cyclohexanedimethanol, pentaerylthritol, pinane, pinaneol, 2,3-cis/exo-pinanediol ([1R,2R,3S,5R]-[-]-pinanediol, and [1S,2S,3R,5S]-[+]-pinanediol]), (1R)-(-)-trans-pinane-1,10-diol, (1S,2S,5S,)-2-hydroxy-3-pinanone, (-)-isopinocampheol, (S)-cis-verbenol, bornane, borneol, 2,3-cis/exo-bornanediol, 2,3-trans-bornanediol, camphanediol, camphenediol, cis-p-menthane-3,8-diol, trans-p-menthane-3,8-diol, sobrerol (trans-p-meth-6-ene-2,8-diol), α-terpineol, terpinen-4-ol, (-)-cis-myrtanol [(1S,2R)-10-Pinanol], (+)-trans-myrtanol [(1R,2R)-10-Pinanol], (-)-trans-myrtanol [(1S,2S)-10-Pinanol], (-)-myrtenal [(1R)-2-Pinen-10-al], (-)-myrtenol [(1R)-2-Pinene-10-ol], carveol {p-mentha-6,8-dien-2-one] and menthol.

12. The composition of claim 11, wherein the compound is selected from 2,3-cis/exo-pinanediol ([1R,2R,3S,5R]-[-]-pinanediol and [1S,2S,3R,5S]-[+]-pinanediol]; 2,3-cis/exo-bornanediol; 5-norbornene-2,2-dimethanol; norbornane-2,2-dimethanol; 2-hydroxy-2-norbornanemethanol; 1-(exo-2-norbornyl-)-propan-1,2-diol; and 1-(endo-2-norbornyl-)-propan-1,2-diol; (1S,2S,5S,)-2-hydroxy-3-pinanone; 2,3-trans-pinanediol; (1R)-(-)-trans-pinane-1,10-diol; 2,3-trans-bornanediol; cis-p-menthane-3,8-diol; trans-p-menthane-3,8-diol; 1,2-cis-cyclopentanediol, 2,3-cis/exo-norbornanediol; 2-norbornanemethanol; (1R)-(-)-myrtenol, and 3,3-dimethyl-1,2-butanediol.

13. The composition of claim 9, wherein the differentiation reverses neuronal damage.

14. The composition of claim 9, wherein the differentiation alleviates a neurodegenerative disease.

15. The composition of claim 14, wherein the disease

is selected from the group consisting of Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, diffuse cerebral cortical atrophy, Lewy-body dementia, Pick disease, mesolimbocortical dementia, thalamic degeneration, Huntington chorea, cortical-striatal-spinal degeneration, cortical-basal ganglionic degeneration, cerebrocerebellar degeneration, familial dementia with spastic paraparesis, polyglucosan body disease, Shy-Drager syndrome, olivopontocerebellar atrophy, progressive supranuclear palsy, dystonia musculcrum deformans, Hallervorden-Spatz disease, Meige syndrome, familial tremors, Gilles de la Tourette syndrome, acanthocytic chorea, Friedreich ataxia, Holmes familial cortical cerebellar atrophy, Gerstmann-Straussler-Scheinker disease, progressive spinal muscular atrophy, progressive balbar palsy, primary lateral sclerosis, hereditary muscular atrophy, spastic paraplegia, peroneal muscular atrophy, hypertrophic interstitial polyneuropathy, heredopathia atactica polyneuritiformis, optic neuropathy, and ophthalmoplegia.

16. The method of claim 9, wherein the differentiation alleviates a cancerous, tumorous or proliferative disorder.

17. A method for increasing the differentiation of mammalian neuronal cells, which comprises administering to a mammal in need of such increase an effective amount of a C_3-C_{50} diol, which may be aliphatic or aromatic, linear, branched, mono-, bi- or polyclicic, saturated or unsaturated, unsubstituted, mono- or polysubstituted.

18. A composition for increasing the differentiation of mammalian neuronal cells, which comprises:

a) an effective amount of a C_3-C_{50} diol, which may be aliphatic or aromatic, linear, branched, mono-, bi- or

polyclicic, saturated or unsaturated, unsubstituted, mono- or polysubstituted; and

b) a suitable carrier.

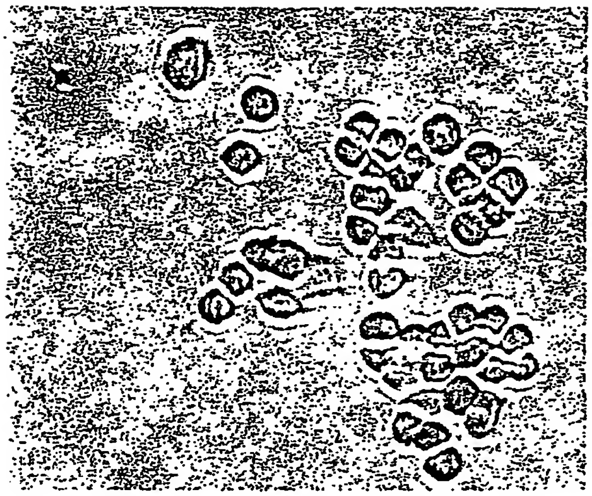


Figure 1A

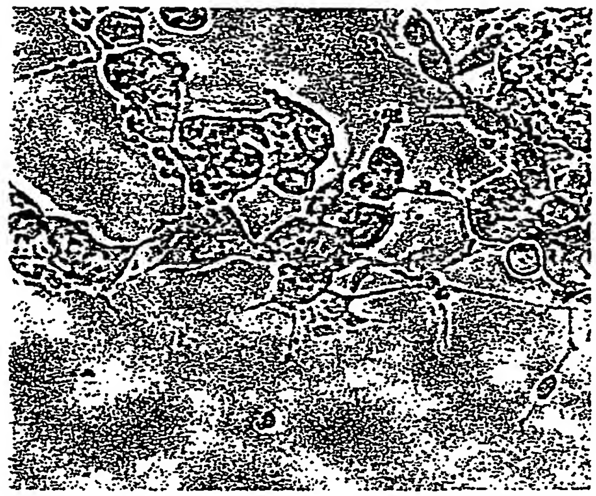


Figure 1B

Figure 2A

Figure 2B

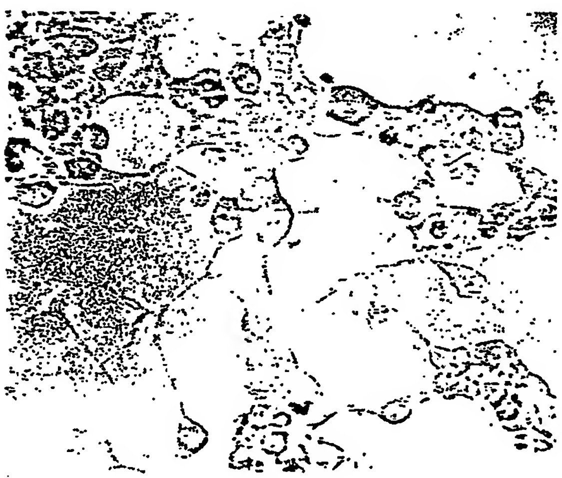


Figure 2C

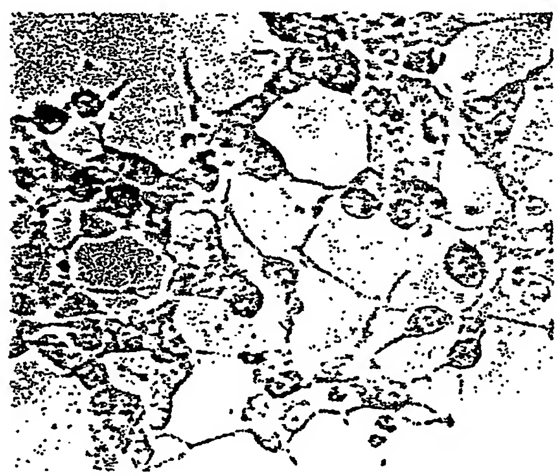


Figure 2D

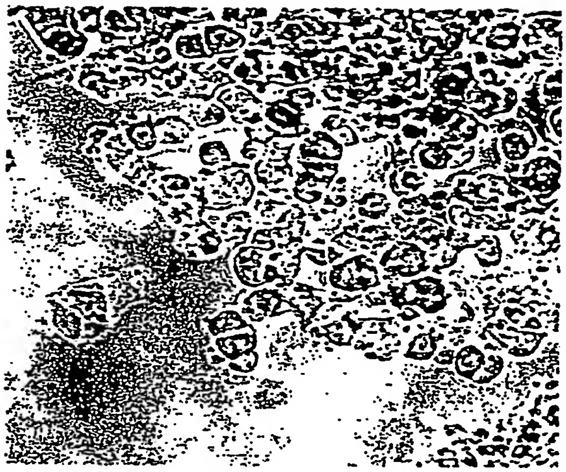


Figure 3A

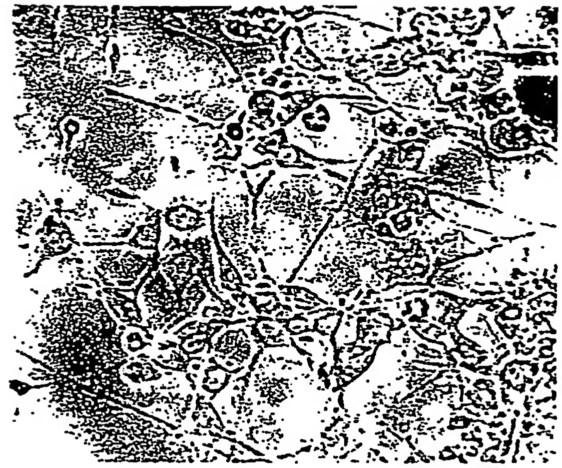


Figure 3B

Figure 3C

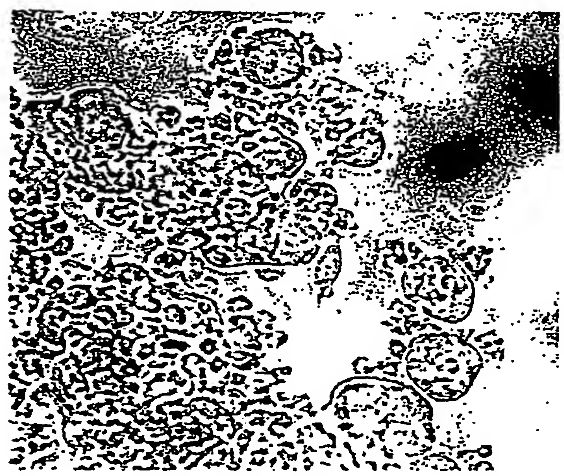


Figure 3D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11840

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 31/045
US CL :514/729, 738, 675, 557
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/729, 738, 675, 557

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CHEMICAL ABSTRACTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,210,076 A (BERLINGER et al) 11 May 1993, see entire document.	1-18
A	US 5,352,440 A (GILCHREST et al) 04 October 1994, see entire document.	1-18
A	US 5,532,001 A (GILCHREST et al) 02 July 1996, see entire document.	1-18
A	US 5,554,359 A (FULLER) 10 September 1996, see entire document.	1-18

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 AUGUST 1999	10 SEP 1999

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	JAMES H. REAMER
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235